Dependence of the Enzymatic Velocity on the Substrate Dissociation Rate

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1. INTRODUCTION

Living cells cannot exist without enzymes. These vital proteins are involved in a variety of processes that are critical for cellular function including signaling, energy transduction, cellular transport, and transfer of genetic information via transcription and translation.1,2 Enzymes selectively accelerate biochemical reactions by many orders of magnitude to time scales that allow cells to function normally. Although the general principles of enzyme functioning are known,1,2 the specific details of how they work at the microscopic level are not always available. Simple Michaelis–Menten kinetics assumes that the enzyme–substrate complex has only one conformation that decays as a single exponential. As a consequence, the enzymatic velocity decreases as the dissociation (off) rate constant of the complex increases. Recently, Reuveni et al.3 showed that it is possible for the enzymatic velocity to increase when the off rate becomes higher, if the enzyme–substrate complex has many conformations which dissociate with the same off rate constant. This was done using formal mathematical arguments, without specifying the nature of the dynamics of the enzyme–substrate complex. In order to provide a physical basis for this unexpected result, we derive an analytical expression for the enzymatic velocity assuming that the enzyme–substrate complex has multiple states and its conformational dynamics is described by rate equations with arbitrary rate constants. By applying our formalism to a complex with two conformations, we show that the unexpected off rate dependence of the velocity can be readily understood: If one of the conformations is unproductive, the system can escape from this “trap” by dissociating, thereby giving the enzyme another chance to form the productive enzyme–substrate complex. We also demonstrate that the nonmonotonic off rate dependence of the enzymatic velocity is possible not only when all off rate constants are identical, but even when they are different. We show that for typical experimentally determined rate constants, the nonmonotonic off rate dependence can occur for micromolar substrate concentrations. Finally, we discuss the relation of this work to the problem of optimizing the flux through singly occupied membrane channels and transporters.

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dissociation or by the formation of a product, P. This can be described by the kinetic scheme: 

\[ E + S \stackrel{k_{on}}{\rightleftharpoons} ES \stackrel{k_{cat}}{\rightarrow} E + P \]  

(1)

where \( k_{on} \) is the bimolecular binding rate constant, \( k_{cat} \) is the dissociation or unbinding rate constant, and \( k_{cat} \) is the catalytic rate constant.

When the substrate concentration, \( c \equiv [S] \), is constant, the system comes to a steady-state. The enzymatic or turnover velocity, \( V \), is defined here as the mean number of product molecules generated by a single enzyme per unit time. It is also the inverse of the mean time between successive conversions of the substrate. This velocity, which is essentially an enzymatic reaction rate, is given by the single-molecule version of the Michaelis–Menten formula: 

\[ V_{MM} = \frac{k_{cat}k_{on}c}{k_{cat} + k_{off} + k_{on}c} = k_{cat}c \]

(2)

where \( K_M = (k_{cat} + k_{off})/k_{on} \) is the Michaelis constant. According to this expression, \( V \) monotonically decreases as \( k_{off} \) increases, as one would expect from simple physical considerations.

The Michaelis–Menten kinetic scheme, eq 1, assumes that the enzyme–substrate complex has only one conformation. Arguably the simplest generalization of this scheme is to allow just the enzyme–substrate complex to have many conformations. Using the sophisticated probabilistic arguments, it has recently been shown that if each and every conformation of the complex has the same dissociation rate constant, \( k_{on} \), and the conformations of free enzyme interconvert on a time scale that is much shorter than its mean lifetime, then the enzymatic velocity can be written as

\[ V_{GMM} = \frac{\hat{f}_{cat}(k_{off})}{(k_{off})^{-1} + [1 - \hat{f}_{cat}(k_{off})]/k_{off}} \]

(3)

independent of the nature of the enzyme–substrate complex dynamics. Here \( f_{cat}(k_{off}) \) is the Laplace transform of function \( f_{cat}(t) : f_{cat}(k_{off}) = \int f_{cat}(k_{off}) \exp(-k_{off} t) dt \). When \( f_{cat}(t) = k_{cat} \exp(-k_{off} t) \), eq 3 reduces to eq 2.

When \( f_{cat}(t) \) is more complex than a single exponential and the unbinding time is exponentially distributed and statistically independent of the catalytic time, Reuveni et al. recently "demonstrated the feasibility of an effect previously conceived to be impossible—the acceleration of enzymatic reactions via increasing the substrate unbinding rate." In other words, it was shown that \( V_{GMM} \) can be a nonmonotonic function of \( k_{off} \), i.e., it first grows with \( k_{off} \) reaches a maximum, and then goes to zero, as \( k_{off} \rightarrow \infty \). This result was obtained using mathematical arguments, without specifying the nature of the underlying dynamics of the enzyme–substrate complex.

The goal of this article is to provide a physical understanding of what is, at first sight, a remarkable result and to see how robust this effect is (i.e., what happens when the off-rates differ). We first derive an analytical expression for the turnover velocity in a model where the conformations of the enzyme–substrate complex are discrete and interconvert according to the rate equations of ordinary chemical kinetics, assuming that the on, off, and catalytic rate constants are arbitrary. When the \( k_{off} \)'s are the same for all enzyme–substrate conformations, we recover eq 3. In this way, we can make the definition of the function \( f_{cat}(t) \) more precise, by establishing the connection between \( f_{cat}(t) \) and the enzyme–substrate complex dynamics. We show that \( f_{cat}(t) \) is the probability density of the lifetime of the enzyme–substrate complex in the hypothetical case that all unbinding rate constants are zero, when different conformations of the complex are initially populated in proportion to the individual binding rate constants. It is important to note that this is not the probability density of the lifetime of the enzyme–substrate complex on condition that it decays via the catalytic channel. We then apply our general formula to simple kinetic schemes involving just two enzyme–substrate conformations. Our physical insight into how the increasing unbinding rate can accelerate the turnover is that if one of the conformations of the enzyme–substrate complex is catalytically inactive, then one way of escaping such a "trap" is for the substrate to dissociate. Thus, one can readily imagine that in certain cases, increasing the unbinding rate can increase the enzymatic turnover velocity.

2. ENZYME TURNOVER VELOCITY

We begin by deriving an expression for the steady-state enzymatic reaction rate for a simple generalization of the Michaelis–Menten model. Although the derivation is elementary in the sense that it involves only algebra, it may be skipped, and the reader can proceed to the discussion of the kinetic schemes shown in Figure 1.

The free enzyme is assumed to have a single conformation (or equivalently many conformations that interconvert sufficiently quickly). The enzyme–substrate complex has \( N \) discrete conformations \( E_S_i \), \( i = 1, 2, \ldots, N \). The dynamics is assumed to be Markovian and the interconversion of the enzyme–substrate conformations is described by a rate matrix \( K \). Its nondiagonal element \( K_{ij} \) is the rate constant for the transitions \( E_S_i \rightarrow E_S_j, i \neq j \), and its diagonal elements \( K_{ii} = -\sum_{j \neq i} K_{ij} \) because of probability conservation. Thus, we have \( \sum_{j = 1}^{N} K_{ij} = 0 \) for \( j = 1, 2, \ldots, N \). In matrix notation this can be written as \( I^T K = 0 \), where \( I^T \) is a row vector with all elements equal to unity and 0 is a column vector with all zero elements.

When the substrate concentration, \( c \), is a constant then the substrate binding is pseudo-first order and the transition \( E \rightarrow E_S \) is described by the pseudo-first order rate constant \( k_{on}(c) \). The dissociation of the complex \( E_S \), \( E_S \rightarrow E + S \), is described by the rate constant \( k_{off}(c) \). Finally, the rate constant for the conversion of \( E_S \) to product, \( E_S \rightarrow E + P \), is \( k_{cat}(c) \).

The turnover velocity can be expressed in terms of the steady-state probabilities of the enzyme–substrate conformations, \( p_i, i = 1, 2, \ldots, N \), as

\[ V = \sum_{i = 1}^{N} k_{off}(c) p_i = I^T K_{cat} p \]

(4)

where \( K_{cat} \) is a diagonal matrix with elements \( k_{cat}(c) \) on the diagonal and \( p \) is a column vector with elements \( p_i \). If we denote the steady-state probability of finding the enzyme to be free by \( p_0 \), the normalization condition for all probabilities can be written as

\[ p_0 + \sum_{i = 1}^{N} p_i = 1 = R_0 + I^T p \]

(5)

The steady-state probabilities satisfy
for \( i = 1, 2, \ldots, N \). This is just a statement of the balance of the steady-state probability fluxes. If we introduce the diagonal matrix \( \mathbf{K}_{\text{off}} \) with elements \( k_{\text{off}}(i) \) on the diagonal and the column vector \( \mathbf{k}_{\text{on}} \) with elements \( k_{\text{on}}(i) \), then eq 6 can be written in matrix notation as

\[
\sum_{i=1}^{N} K_{i,j}p_{j} - [k_{\text{off}}(i) + k_{\text{cat}}(i)]p_{i} + c_{k_{\text{on}}(i)p_{0}} = 0
\]

Solving this for vector \( \mathbf{p} \), we have

\[
\mathbf{p} = (\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0}
\]

Substituting this into eq 5 and solving the resulting equation for \( p_{0} \), we obtain

\[
p_{0} = \frac{1}{1 + \mathbf{1}^{T}(\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0}
\]

Finally, by substituting the vector \( \mathbf{p} \) in eq 8 with \( p_{0} \) given by eq 9 into eq 4, we find that the turnover velocity is

\[
V = \frac{\mathbf{1}^{T}\mathbf{K}_{\text{cat}}(\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0}}{1 + \mathbf{1}^{T}(\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0}}
\]

This can be rewritten in the form of the Michaelis–Menten equation (see eq 2) with \( \mathbf{K}_{M} = 1/(\mathbf{1}^{T}(\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0}) \), \( \mathbf{k}_{\text{cat}} = \mathbf{K}_{M}\mathbf{1}^{T}(\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0} \). This is a special case of more complex expressions for \( \mathbf{K}_{\text{cat}} \) and \( \mathbf{k}_{\text{cat}} \) recently obtained by Barel et al.\(^{11} \) (see their eq 26) in the context of enzymatic modification of DNA.

Let us present the result in eq 10 in a physically appealing form. Dividing the numerator and denominator of this equation by \( \mathbf{1}^{T}\mathbf{k}_{\text{on}}f_{0} = \sum_{i=1}^{N} k_{\text{on}}(i)c_{i} \), we obtain

\[
V = \frac{\Phi_{\text{cat}}}{\tau_{\text{ES}}} \quad (11)
\]

where we have defined:

\[
\tau_{\text{ES}} = (\mathbf{1}^{T}\mathbf{k}_{\text{on}}f_{0})^{-1} = \left( \sum_{i=1}^{N} k_{\text{on}}(i)c_{i} \right)^{-1}
\]

\[
\Phi_{\text{cat}} = \frac{\mathbf{1}^{T}(\mathbf{K}_{\text{off}} + \mathbf{K}_{\text{cat}} - \mathbf{K})^{-1}\mathbf{k}_{\text{on}}f_{0}}{\mathbf{1}^{T}\mathbf{k}_{\text{on}}f_{0}}
\]

The crucial point is that these three quantities turn out to have simple physical interpretations. Clearly, \( \tau_{\text{ES}} \) is just the mean lifetime of the free enzyme. It can be shown by direct calculation (see Appendix A) that \( \tau_{\text{ES}} \) is the mean lifetime of the enzyme–substrate complex in which the states were initially occupied in proportion to their on rates (i.e., the probability of forming a new complex in the conformation ES). Finally, it can be also shown (see Appendix A) that \( \Phi_{\text{cat}} \) is the probability that the enzyme–substrate complex decays through the catalytic channel rather than by dissociating (also known as the splitting or commitment probability). Eq 11 is similar to eq 11 of ref 8 even though the notations are quite different.

Now that we understand the physical meaning of \( \tau_{\text{ES}} \) and \( \Phi_{\text{cat}} \), eq 11 for the enzymatic velocity becomes transparent. The sum of the mean lifetimes in the denominator, \( \tau_{\text{on}} + \tau_{\text{ES}} \), is the mean time between two successive binding events leading to the formation of the enzyme–substrate complex. The inverse of this mean time is the mean number of the enzyme–substrate complexes formed by the single enzyme per unit time. Each complex either decays through the catalytic channel forming a product or dissociates. These two outcomes occur with the probabilities \( \Phi_{\text{cat}} \) and \( (1-\Phi_{\text{cat}}) \), respectively. The product of \( \Phi_{\text{cat}} \) and the mean number of complexes formed per unit time, given on the right side of eq 11, is the mean number of product molecules generated by the single enzyme per unit time. This mean number is, by definition, the enzymatic turnover velocity \( V \).

Let us now show that eq 11 reduces to eq 3 when the dissociation or unbinding rate constant of every enzyme–substrate conformational state is equal to \( k_{\text{off}} \). In this case, the survival probability of the enzyme–substrate complex, \( S_{\text{ES}}(t) \), factors,

\[
S_{\text{ES}}(t) = e^{-k_{\text{off}}S_{\text{cat}}(t)}
\]

where \( S_{\text{ES}}(t) \) is the survival probability of the enzyme–substrate complex when \( k_{\text{off}} = 0 \). The lifetime probability density of this (hypothetical) complex is \( f_{\text{cat}}(t) \equiv -dS_{\text{cat}}(t)/dt \). Therefore, the lifetime of the enzyme–substrate complex, \( \tau_{\text{ES}} \), can be written as

\[
\tau_{\text{ES}} = \int_{0}^{\infty} S_{\text{ES}}(t)dt = \int_{0}^{\infty} e^{-k_{\text{off}}S_{\text{cat}}(t)}dt = \frac{\dot{S}_{\text{cat}}(k_{\text{off}})}{S_{\text{cat}}(k_{\text{off}})} \quad (14)
\]

Finally, \( \Phi_{\text{cat}} = 1 - \Phi_{\text{diss}} \) where \( \Phi_{\text{diss}} \) is the probability that the enzyme–substrate complex decays by dissociation. It is given by eq 12c with \( \mathbf{k}_{\text{cat}} \) and \( \mathbf{k}_{\text{off}} \) interchanged. When \( \mathbf{k}_{\text{off}} = \mathbf{k}_{\text{cat}} \mathbf{I} \), it follows from eq 12b that \( \mathbf{Phi}_{\text{cat}} = \mathbf{k}_{\text{off}}S_{\text{ES}} \) and thus

\[
\Phi_{\text{cat}} = 1 - k_{\text{off}}\tau_{\text{ES}} = 1 - k_{\text{off}}S_{\text{cat}}(k_{\text{off}}) = \dot{f}_{\text{cat}}(k_{\text{off}})
\]

Substituting eqs 12a, 14, and 15 into eq 11, we recover eq 3 if we identify \( \mathbf{k}_{\text{cat}} \) with \( \Sigma_{i=1}^{N} k_{\text{on}}(i) \).

3. TWO-STATE ES-COMPLEXES

We will now apply the above formalism to a variety of simple kinetic schemes shown in Figure 1. These were chosen so that one can readily understand the physical reason why the turnover velocity can sometimes increase with increasing unbinding rates. In scheme A the substrate binds to the enzyme forming the complex only in state 1. This state can decay via the dissociation or catalytic channels or be converted to the state 2. The rate constants characterizing these three processes are \( k_{\text{off}} \), \( k_{\text{cat}} \), and \( \alpha \), respectively. The second state is unproductive and can decay only by dissociating with the rate constant \( k_{\text{diss}} \) thereby allowing the enzyme to try again. Since state 2 is a trap where the product cannot form, the overall reaction rate can be increased by accelerating the rate of unbinding. However, too large unbinding rates will eventually slow down the reaction rate by decreasing the lifetime of the productive state 1. Using the above formalism, it is straightforward to show that for this model the turnover velocity is

\[
V = \frac{\dot{f}_{\text{cat}}k_{\text{cat}}k_{\text{on}}f_{0}}{k_{\text{off}}(k_{\text{off}} + k_{\text{cat}} + \alpha) + (k_{\text{off}} + \alpha)k_{\text{on}}f_{0}}
\]
velocity to increase with $k_{\text{off}}$. At the same time, this example is unphysical because detailed balance is violated for substrate binding and conformational dynamics.

The simplest scheme which satisfies the condition of detailed balance is shown in Figure 1B. In this case, increasing $k_{\text{off}}$ cannot always increase $V$ because, for example, when $\alpha \to \infty$ this scheme reduces to the classical Michaelis–Menten scheme. Utilizing the above formalism it can be shown that the turnover velocity for this model is given by

$$V = \frac{k_{\text{cat}}(k_{\text{off}} + 2\alpha)k_{\text{cat}}}{k_{\text{on}}^2 + k_{\text{off}}(k_{\text{cat}} + 2\alpha) + \alpha k_{\text{cat}}^2 + (k_{\text{cat}} + 2k_{\text{off}} + 4\alpha)k_{\text{cat}}^2}. \quad (17)$$

This velocity is a nonmonotonic function of $k_{\text{off}}$ only when the rate constants satisfy $k_{\text{cat}}k_{\text{cat,c}} > \alpha(k_{\text{cat}} + 4\alpha)$. For fixed values of the rate constants this inequality can always be satisfied for sufficiently large concentration $c$. However, when the interconversion between two enzyme–substrate states is very fast ($\alpha \to \infty$) this inequality can never be satisfied and nothing unusual happens, as to be expected, since in this limit the model reduces to the Michaelis–Menten one. The results for this kinetic scheme are illustrated in Figure 3. The nonmonotonic behavior can only be observed for concentrations above $c = 1.8$ $\mu$M for the utilized rate constants.

Finally, we show that the enzymatic velocity may be a nonmonotonic function of the unbinding rate even when the off rate constants of the two states of the enzyme–substrate complex are different. The simplest such scheme is shown in Figure 1C. For substrate binding to satisfy the condition of detailed balance, the rate constants must satisfy $\alpha k_{\text{off}}(2) = \beta k_{\text{off}}(1)$. For fixed rates $\alpha$ and $\beta$, this relates the off rate constants, $k_{\text{off}}(1)$ and $k_{\text{off}}(2)$. Using this to eliminate $k_{\text{off}}(2)$, the enzymatic velocity can be written as

$$V = \frac{k_{\text{cat}}(k_{\text{off}}(1) + 2\alpha)k_{\text{cat}}c}{[k_{\text{off}}(1) + k_{\text{off}}(2)](k_{\text{cat}} + 2\alpha) + \alpha k_{\text{cat}}^2 + (\alpha + \beta)(k_{\text{off}}(1) + 2\alpha) + \alpha k_{\text{cat}}|k_{\text{cat,c}}|. \quad (18)$$

When $\alpha = \beta$ this reduces to eq 2 since the two off rate constants must be equal for the condition of detailed balance to be satisfied. The enzymatic velocity given by eq 18 is a nonmonotonic function of $k_{\text{off}}(1)$ only when the rate constants satisfy $k_{\text{cat}}k_{\text{cat,c}} > \beta(k_{\text{cat}} + 4\alpha)$. For fixed values of the rate constants this inequality can always be satisfied for sufficiently large concentration $c$. Thus, the turnover velocity can increase with increasing unbinding rates even when eq 3 is inapplicable because $k_{\text{off}}(1) \neq k_{\text{off}}(2)$.

The nonmonotonic dependence of the enzymatic velocity on the unbinding rate may be observed only when the substrate concentration is sufficiently high. The inequalities, mentioned above, provide constraints on this concentration. For kinetic
scheme shown in Figure 1C, the concentration must satisfy $c > (1 + 4\alpha/k_{\text{cat}})\beta/k_{\text{cat}}$. One can see that the concentration on the right-hand side of this inequality decreases as the rate constants $k_{\text{cat}}$ and $k_{\text{on}}$ increase and the rate constants $\alpha$ and $\beta$ decrease. When $k_{\text{cat}} \gg \alpha$, the inequality simplifies to $c > \beta/k_{\text{cat}}$. As an example, let us consider rates that are typical for dihydrofolate reductase which catalyzes the reduction of 7,8-dihydrofolate to produce 5,6,7,8-tetrahydrofolate, where $k_{\text{cat}}$ is of the order $10^6-10^7$ M$^{-1}$ s$^{-1}$, $\beta$ is of the order of 10 s$^{-1}$. For these parameter values and the kinetic scheme shown in Figure 1C, the concentrations must be higher than 1–10 $\mu$M. This estimate shows that the nonmonotonic dependence of the enzymatic velocity on the unbinding rate can occur in the physically relevant concentration range.

4. CONCLUDING REMARKS

In this article we consider the turnover velocity of a single enzyme assuming that the enzyme–substrate complex has $N$ discrete conformations, and its dynamics is described by Markovian rate equations. We derive an expression for the enzymatic velocity, eqs 10–12c, which generalizes the Michaelis–Menten formula, eq 2, to the case when the enzyme–substrate complex has $N$ conformational states. Two-state ES-complexes are used to shed light on the counterintuitive nonmonotonic dependence of the velocity on the dissociation rate.8,9 We show that such a dependence may arise when there are unproductive conformations of the enzyme–substrate complex. By accelerating escape from such conformations by dissociation, the system can increase its enzymatic velocity.

The main limitation of this work is the assumption that the conformations of the free enzyme interconvert sufficiently rapidly so that the free enzyme is always in conformational equilibrium. This allows one to treat the free enzyme as a single conformation. When this assumption is relaxed, the problem becomes more complex, and the dependence of the enzymatic velocity on the substrate concentration is in general no longer hyperbolic, as expected from the Michaelis–Menten equation. It is likely that the nonmonotonic dependence of the enzymatic velocity on the unbinding rate exists for such a model in a certain region of parameter space, but this remains to be investigated.

Our expression for the enzymatic velocity can also be used to study unidirectional membrane transport by singly occupied channels and transporters. For example, the transport through a two-site channel or by a transporter with two conformations can be described by a kinetic scheme analogous to that shown in Figure 1D when there are no molecules on the right side of the membrane. In such systems the unidirectional flux of the transported species shows nonmonotonic dependence on $k_{\text{off}}$ only when $k_{\text{cat}}$ and $k_{\text{off}}$ are correlated. If only $k_{\text{off}}$ is changed, keeping all other parameters fixed, the flux will be a monotonic function of $k_{\text{off}}$. Finally, we note that the above derivation of the expression for the turnover velocity is actually more general than it would appear at first sight. If we would have assumed that the enzyme–substrate conformations are continuous rather than discrete, all we would have to do is to replace summation by integration and inverse matrices by the appropriate Green’s functions.

APPENDIX A

Derivation of Eqs 12b and 12c

Consider the decay of an enzyme-substrate complex formed at $t = 0$. Let $P(t)$ be an $N$-state column vector with elements $P_i(t)$ which are the probabilities of finding the enzyme-substrate complex in state $i$ at time $t$, $i = 1, 2, \ldots, N$, when $k_{\text{on}} = 0$. This vector satisfies the irreversible rate equation

$$\frac{dP(t)}{dt} = (K - K_{\text{off}} - K_{\text{cat}})P(t) \tag{A.1}$$

subject to the initial condition $P(0) = k_{\text{on}}/(1^T k_{\text{on}})$. The survival probability of the complex at time $t$, $S_{ES}(t)$, is the sum of the probabilities $P_i(t)$,

$$S_{ES}(t) = \sum_{i=1}^{N} P_i(t) = 1^T P(t) \tag{A.2}$$

As follows from eqs A.1 and A.2, the probability density of the lifetime of the enzyme-substrate complex is $-dS_{ES}/dt = 1^T (K_{\text{off}} + K_{\text{cat}})P(t)$, where we have used the fact that because of the probability conservation $1^T K = 0$. This probability density is the sum of two contributions corresponding to the enzyme-substrate complex decaying via the dissociation and catalytic channels.

The mean lifetime, $\tau_{ES}$, of the complex is

$$\tau_{ES} = \int_0^\infty (-dS_{ES}/dt)dt = \int_0^\infty S_{ES}(t)dt = 1^T \hat{P} \tag{A.3}$$

where the vector $\hat{P}$ is defined by

$$\hat{P} = \int_0^\infty P(t)dt \tag{A.4}$$

To find the equation satisfied by the vector $\hat{P}$, we integrate both sides of eq A.1 with respect to time from zero to infinity. In this way we obtain

$$(K_{\text{off}} + K_{\text{cat}} - K)\hat{P} = (0) = k_{\text{on}}/(1^T k_{\text{on}}) \tag{A.5}$$

where we have used the relation $\int_0^\infty (dP(t)/dt)dt = P(\infty) = P(0) - k_{\text{off}}/(1^T k_{\text{on}})$. The formal solution to eq A.4 is

$$\hat{P} = (K_{\text{off}} + K_{\text{cat}} - K)^{-1} k_{\text{on}}/(1^T k_{\text{on}}) \tag{A.6}$$

Substituting this into eq A.3, we arrive at the expression for $\tau_{ES}$ in eq 12b.

As mentioned earlier, the decay rate of the survival probability of the complex at time $t$ via the catalytic channel is given by $1^T K_{\text{cat}} P(t)$. The probability $\Phi_{\text{cat}}$ that the enzyme-substrate complex decays through the catalytic channel, is the integral of this rate with respect to time from zero to infinity,

$$\Phi_{\text{cat}} = \int_0^\infty 1^T K_{\text{cat}} P(t)dt = 1^T K_{\text{cat}} \hat{P} \tag{A.7}$$

Using eq A.6 for $\hat{P}$, we recover the expression for $\Phi_{\text{cat}}$ in eq 12c.

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